PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 153460-1 CC	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/IL2004/000692	International filing date *(day/month/year)* 28 July 2004 (28.07.2004)	Priority date *(day/month/year)* 28 July 2003 (28.07.2003)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

   - ☒ Box No. I — Basis of the report
   - ☒ Box No. II — Priority
   - ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV — Lack of unity of invention
   - ☒ Box No. V — Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☒ Box No. VI — Certain documents cited
   - ☐ Box No. VII — Certain defects in the international application
   - ☐ Box No. VIII — Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 30 January 2006 (30.01.2006)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 740 14 35	Authorized officer Simin Baharlou Telephone No. +41 22 338 71 30

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

3|2

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Applicant's or agent's file reference see form PCT/ISA/220	Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet) **FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/IL2004/000692	International filing date *(day/month/year)* 28.07.2004
Priority date *(day/month/year)* 28.07.2003	
International Patent Classification (IPC) or both national classification and IPC C12Q1/68	
Applicant YISSUM RESEARCH DEVELOPMENT COMPANY OF THE ...	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized Officer

Bellmann, A

Telephone No. +31 70 340-8958

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IL2004/000692

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IL2004/000692

Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4,6,11-20,22,29-31,33-36
	No:	Claims	1-3,5,7-10,21,23-28,32
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-36
Industrial applicability (IA)	Yes:	Claims	1-36
	No:	Claims	-

2. Citations and explanations

   see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

   and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

   see form 210

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents are referred to in this communication:

D1: US 6 326 144 (Bawendi *et al.*, 2001)
D2: WILLARD D M ET AL: "CdSe-ZnS quantum dots as resonance energy transfer donors in a model protein-protein binding assay" NANO LETTERS, AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC, US, vol. 1, no. 9, 2001, pages 469-474
D3: US 5 945 283 (Kwok *et al.*, 1999)
D4: DE 101 17 866 (GAUB, 2002)
D5: US 5 856 096 (Windle *et al.*, 1999)
D6: UEHARA H ET AL:" Detection of Telomerase Activity Utilizing Energy Transfer Primers: Comparison with Gel- and ELISA-Based Detection." BIOTECHNIQUES, vol. 26, pages 552-558

1 **NOVELTY** (Article 33(2) PCT)

1.1 D1 discloses a method for determining an analyte in an assayed sample, comprising:
(a) providing a semiconductor nanoparticle carrying a recognition agent capable of specifically binding to the analyte, namely a quantum dot carrying a DNA probe specifically binding to the analyte, i.e. target nucleic acid (cf. col.14, par.2)
(b) contacting said semiconductor nanoparticles with the assayed sample
(c) providing an acceptor capable of immobilization directly or indirectly, in the presence of the analyte, to the recognition agent, wherein the acceptor is a DNA probe labelled with a fluorescence tag or with a differently sized quantum dot (cf. col.14, par.2 and par.3)
(d) providing assay conditions, such that in the presence of the target nucleic acid in the assayed sample a reaction would occur, resulting in the indirect immobilization of the acceptor to the recognition agent, i.e. binding of both DNA probes to the target nucleic acid
(e) irradiating the system so as to cause excitation of the nanoparticles and energy transfer to the acceptor; and generation of an electromagnetic signal,
(f) detecting said signal, whereby the signal is indicative of the presence and

amount of said analyte in the sample (cf. col.14, par.2).
Hence, the subject-matter of **independent claim 1 and dependent claims 2,3,5,7 to 10 and 32** is not novel over D1 (Article 33(2) PCT).

1.2 D2 discloses a sensing device for determining a specific analyte in an assayed sample, the device comprising assay unit, namely a quartz cuvette comprising a system of semiconductor nanoparticles carrying a recognition agent, i.e. quantum dots with bound BSA and an acceptor, i.e. the fluorophor TMR bound to streptavidin.
Hence, the subject-matter of **independent claim 21 and dependent claims 23 to 28** is not novel over D2 (Article 33(2) PCT).

2 **INVENTIVE STEP** (Article 33(3) PCT)

2.1 Document D3 is considered to represent the most relevant state of the art for **claim 12** in its present form. D3 discloses a method for determining an analyte in an assayed sample, comprising:
(a) providing fluorescein-labelled recognition agent capable of specifically binding to the analyte, namely a primer specifically annealing adjacent to a SNP (cf. Fig.1 and col.5)
(b) contacting said recognition agent with the assayed sample
(c) providing an acceptor capable of immobilization directly or indirectly, in the presence of the analyte, to the recognition agent, wherein the acceptor is Rox-labelled ddCTP
(d) providing assay conditions, such that in the presence of the analyte in the assayed sample a reaction would occur, resulting in the direct immobilization of the acceptor to the recognition agent, i.e. incorporation of the ddCTP in the primer by DNA polymerase
(e) irradiating the system so as to cause excitation of the fluorescein (donor) and energy transfer to the acceptor; and generation of an electromagnetic signal,
(f) detecting said signal, whereby the signal is indicative of the presence of said analyte in the sample (cf. Fig.1 and col.5).

2.2 The subject-matter of claim 12 differs from the subject-matter disclosed in closest prior art document D3 in that the recognition agent is labelled with a semiconductor nanoparticle, which acts as an energy donor in the energy transfer.

2.3 No unexpected technical effect appears to be associated with said difference.

2.4 The technical problem to be solved may therefore be regarded as providing an alternative energy donor for a method for determining a SNP in an assayed sample. The proposed solution is to use semiconductor nanoparticles.

2.5 This solution cannot be considered as involving an inventive step for the following reasons:

2.5.1 It is well-known in the state of the art that semiconductor nanoparticles, i.e. quantum dots can be used as fluorescence labels for the detection of biomolecules by fluorescence resonance energy transfer (FRET) (cf. D1, col.14, par.2 and 3, D2, whole document, D4, cl.1,31,33, par.5,8,92). To use semiconductor nanoparticles represents merely one of several straightforward possibilities from which the skilled person would select, without the exercise of inventive skill, when searching for alternative energy donors.

2.6 Hence, the subject-matter of **dependent claim 12** does not involve an inventive step (Article 33 (3) PCT).

2.7 Document D5 is considered to represent the most relevant state of the art for claim 18 in its present form. D5 discloses a method for determining an analyte in an assayed sample, comprising:
(a) providing a single stranded DNA recognition agent, that serves as a primer for telomerase reaction (cf. col.3, par.4 and Fig.5)
(b) providing an assay sample comprising cellular extract from one or more cells comprising telomerases (cf. col.2, par.4, col.3 par.2)
(c) contacting said recognition agent with the assayed sample;
(d) providing nucleotide bases including BrdUTP
(e) detecting the incorporation of BrdUTP by a labelled anti-BrdUTP antibody.

2.8 The subject-matter of claim 18 differs from the subject-matter disclosed in closest prior art document D5 in that the telomerase primer is labelled with a semiconductor nanoparticle and that the nucleotide bases are bound to an acceptor, wherein the nanoparticle acts as an energy donor in the energy transfer.

2.9 No unexpected technical effect appears to be associated with said difference.

2.10 The technical problem to be solved may therefore be regarded as providing an alternative detection method for detecting incorporated nucleotide bases. The proposed solution is to use a recognition agent that is labelled with a semiconductor nanoparticle and nucleotide bases that are bound to an acceptor, wherein the nanoparticle acts as an energy donor in the energy transfer.

2.11 This solution cannot be considered as involving an inventive step for the following reasons:

2.11.1 It is well-known that FRET can be used to detect the incorporation of nucleotide bases (cf. D5, Fig.1 and col.5). As the use of semiconductor nanoparticles, i.e. quantum dots as energy donors in FRET is not inventive for the reasons stated above (cf. section 2.5), the use of FRET based on the interaction of semiconductor nanoparticles with energy acceptors represents merely one of several straightforward possibilities from which the skilled person would select, without the exercise of inventive skill, when searching for alternative detection methods for detecting incorporated nucleotide bases.

2.12 Hence, the subject-matter of **dependent claim 18** does not involve an inventive step (Article 33 (3) PCT).

2.13 Document D6 is considered to represent the most relevant state of the art for claim 19 in its present form. D6 discloses a method for determining an analyte in an assayed sample, comprising:
(a) providing a single stranded DNA recognition agent, that serves as a primer for telomerase reaction (cf. Fig.1 and p.553)
(b) providing an assay sample comprising cellular extract from one or more cells comprising telomerases (cf. p.553, col.1, par.3)
(c) contacting said recognition agent with the assayed sample in the presence of nucleotide bases
(d) providing assay conditions enabling telomerase catalyzed DNA elongation reaction thereby producing telomere repeat units bound to said primer,
(e)providing a nucleotide sequence being complementary to the telomere repeat units and being bound to an acceptor and a donor,
(f) providing assay conditions giving rise to a hybridization reaction such that said nucleotide sequence of step (e) may bind to the telomere repeat units;
(g) irradiating the system so as to cause excitation of the donor, transfer of

resonance energy from said nanoparticles to said acceptor and generation of a signal, and
(h) detecting said signal, whereby the signal is indicating the presence and/or amount of telomerase in the sample.

2.14 The subject-matter of claim 19 differs from the subject-matter disclosed in closest prior art document D6 in that the telomerase primer is labelled with a semiconductor nanoparticle acting as the donor and that the probe is labelled only with an acceptor.

2.15 No unexpected technical effect appears to be associated with said differences.

2.16 The technical problem to be solved may therefore be regarded as providing an alternative detection method for detecting a nucleotide sequence. The proposed solution is to use a telomerase primer that is bound to a semiconductor nanoparticle and a probe that is labelled only with an acceptor.

2.17 This solution cannot be considered as involving an inventive step for the following reasons:

2.17.1 It is well-known that FRET between semiconductor nanoparticles and an acceptor (fluorophors or an additional semiconductor nanoparticle), can be used to detect the nucleotide sequences by hybridization, using two probes carrying donor and acceptor molecules (cf. D1, col.14, par.2 and 3). To use a primer with a bound semiconductor nanoparticle and a probe labelled with an acceptor, represents merely one of several straightforward possibilities from which the skilled person would select, without the exercise of inventive skill, when searching for alternative detection methods for detecting a nucleic acid sequence.

2.18 Hence, the subject-matter of **dependent claim 19** does not involve an inventive step (Article 33 (3) PCT).

2.19 The subject-matter of dependent claim 20 differs from the method disclosed in D6 (cf. section 2.13) only in that the probe (cf. cl.20, (c)) carries semiconductor particles as donor and acceptor. To use semiconductor particles as energy donor and acceptor does not provide the basis for an inventive step. The same reasoning applies as for claim 12 (cf. section 2.5).
Hence, the subject-matter of **dependent claim 20** does not involve an inventive

step (Article 33 (3) PCT).

2.20 **Dependent claims 4,6,11,13-17,29-31 and 33 to 36** do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, as all the additional features fall within the scope of customary practise (Article 33(3) PCT).

Re Item VI
Certain document cited

3 Certain published document:

D7: PATOLSKY FERNANDO ET AL: "Lighting-up the dynamics of telomerization and DNA replication by CdSe-ZnS quantum dots." JOURNAL OF THE AMERICAN CHEMICAL SOCIETY. 19 NOV 2003, vol. 125, no. 46, 19 November 2003 (2003-11-19), pages 13918-13919

3.1 Should the priority of the application prove to be invalid, claims 1 to 36 would not appear to be novel over D4 (Article 33(2) PCT).